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REVIEW

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Tolerance to noninherited maternal antigens, reproductive microchimerism and regulatory T cell memory: 60 years after 'Evidence for actively acquired tolerance to Rh antigens'

Jeremy M. Kinder[a], Tony T. Jiang[a], James M. Ertelt[a], Lijun Xin[a], Beverly S. Strong[b], Aimen F. Shaaban[b], and Sing Sing Way[a]

[a]Division of Infectious Diseases and Perinatal Institute, Cincinnati Children's Hospital, Cincinnati, OH, USA; [b]Center for Fetal Cellular and Molecular Therapy, Cincinnati Children's Hospital, Cincinnati, OH, USA

ABSTRACT

Compulsory exposure to genetically foreign maternal tissue imprints in offspring sustained tolerance to noninherited maternal antigens (NIMA). Immunological tolerance to NIMA was first described by Dr. Ray D. Owen for women genetically negative for erythrocyte rhesus (Rh) antigen with reduced sensitization from developmental Rh exposure by their mothers. Extending this analysis to HLA haplotypes has uncovered the exciting potential for therapeutically exploiting NIMA-specific tolerance naturally engrained in mammalian reproduction for improved clinical outcomes after allogeneic transplantation. Herein, we summarize emerging scientific concepts stemming from tolerance to NIMA that includes postnatal maintenance of microchimeric maternal origin cells in offspring, expanded accumulation of immune suppressive regulatory T cells with NIMA-specificity, along with teleological benefits and immunological consequences of NIMA-specific tolerance conserved across mammalian species.

Introduction, pioneering observations on immunological tolerance by Dr. Ray Owen

Each individual among outbred populations is immunologically unique – defined by inherited maternal and paternal genes that encode distinctive MHC haplotype alleles along with other minor alloantigens. This established definition of immunological identity, with ensuing implications for tolerance based on binary self versus non-self antigen distinction is based on pioneering observations by Dr. Ray D. Owen comparing antigen diversity among fraternal twin cattle. In a seminal paper published in 1945, only 5 paragraphs were needed to articulate and re-conceptualize foundational concepts regarding immunological identity and tolerance.[1]

Dr. Owen's roots were in farming dairy cattle. In this context, he made the intriguing observation that a majority of fraternal twin cattle had compatible blood types despite a diversity of at least 40 distinct genetically controlled antigens known for this species.[1] This unexpected finding persisted even in cases of superfecundation, involving twins in the same pregnancy sired by genetically distinct fathers. Owen also noted that one bull derived from a fraternal twin litter failed to transmit his phenotypic blood group antigens in up to 20 of his sired next generation progeny. Reflecting on anatomical vascular anastomoses between bovine twin embryos,[2] these observations were pieced together to postulate co-existence of shared blood cells between genetically non-identical twin cattle throughout adult life.[1] More importantly, Owen recognized the revolutionary cross-disciplinary implications of these findings for immunology and genetics, articulating arguably the first definitive example of persistent immunological tolerance to genetically foreign antigens.

Although this seminal characterization of tolerance between fraternal twin cattle establishing the existence of acquired immunological tolerance is most widely recognized, other related contributions have had equally sustained impacts shaping research on immunological responsiveness to developmentally pertinent genetically foreign antigens. This brief review written to commemorate Dr. Owen's 100th birthday contains a snapshot of past and ongoing work on immune tolerance to noninherited maternal antigen directly stemming from

'Evidence for actively acquired tolerance to Rh antigens reported by Owens and colleagues 60 years ago.'[3]

Human immunological tolerance to noninherited maternal antigens

Despite the pervasive immunological implications stemming from tolerance to discordant cells in the somewhat obscure setting of fraternal twinning, far more common is compulsory exposure of each individual during in utero fetal development to genetically foreign maternal cells and tissues that express noninherited maternal antigens (NIMA). Here, Owen was the first to recognize that physiological exposure to discordant maternal antigens in this developmental context can confer sustained immunological tolerance to NIMA in offspring.[3] Investigating the heterogeneity of sensitization to erythrocyte rhesus (Rh) antigen among Rh-negative women during pregnancies with Rh-positive male partners, Owen postulated that early developmental stimulation by Rh antigen among women born to Rh-positive mothers might confer persistent tolerance to Rh sensitization. In other words, exposure to noninherited antigens expressed by the maternal grandmother may have beneficial impacts in women on the outcome of next-generation pregnancies. This hypothesis was addressed by comparing the maternal Rh status of women categorized as either Rh-tolerant or Rh-intolerant following repeated stimulation by concepti bearing this genetically foreign paternal antigen. Remarkably, a significant majority (78%; 32 of 41) of Rh-tolerant women were shown to have Rh-positive mothers, whereas this maternal Rh skewing was eliminated among Rh-intolerant women (48%; 27 of 56 born to Rh-positive mothers).[3] Thus, a critical association between early developmental exposure to Rh antigen for reproductive age women from their own mothers, and protection against Rh sensitization during next generation pregnancies was recognized. Interestingly however, the incidence of erythroblastosis fetalis or newborn hemolytic disease remained similar among Rh-tolerant and Rh-intolerant mothers suggesting NIMA-specific tolerance to this single alloantigen alone was not sufficient to confer survival benefits to next-generation offspring.[3,4]

As the need for immunological tolerance to genetically foreign antigens became increasingly recognized in transfusion medicine and transplantation over the next 30 years, applicability of NIMA-specific tolerance in these clinical contexts has been further evaluated. Functional consequences of NIMA-specific tolerance were demonstrated in individuals who received multiple blood transfusions and normally developed antibodies against almost all HLA alloantigens. Interestingly however, a majority of transfusion dependent individuals broadly exposed to foreign HLA alleles were found to selectively lack antibodies with specificity to NIMA, compared with noninherited paternal antigen (NIPA) HLA haplotypes.[5] Along with critical implications these data have on organ donor selection prior to transplantation, NIMA-specific tolerance was also recognized to protect against allograft rejection following transplantation. In a landmark retrospective analysis of 205 kidney transplant recipients from HLA mis-matched sibling donors, long-term allograft survival was markedly improved if mismatched for NIMA compared to NIPA HLA haplotypes.[6] In fact, 5 and 10 year survival of NIMA-mismatched kidneys was nearly identical to survival rates of HLA-identical grafts. Together, these classical studies not only reaffirm Owen's initial observation of persistent postnatal NIMA-specific tolerance, but also highlight exciting translational opportunities for exploiting tolerance to NIMA naturally imprinted during early development for improved outcomes after transplantation.

More variable degrees of protection have been described after haematopoietic stem cell transplantation exploiting NIMA-specific tolerance to protect against graft-vs.-host disease (GVHD) in HLA-discordant donor-recipient pairings.[7,8] Among HLA-haploidentical sibling-to-sibling donor-recipient pairs, van Rood and colleagues reported a 1.fold9- reduced risk of acute GVHD in NIMA-mismatched compared with NIPA-mismatched bone marrow transplants.[9] Ichinohe and colleagues described 9.fold9- reduced rates of severe grade III/IV acute GVHD after haematopoietic stem cell transplantation between NIMA-mismatched family members.[10] In addition, long-term follow up of the same cohort by Kanda and colleagues revealed immunosuppressive therapy could be successfully withdrawn for NIMA-mismatched transplant recipients with mild GVHD.[11]

Despite these remarkable protective benefits from initial retrospective analysis, individual case reports of prospectively selected NIMA-mismatched donor-recipient pairs showed less promising results. In one case series of 3 patients selected to receive NIMA-mismatched donor haematopoietic stem cells,

acute GVHD occurred in 2 recipients and graft rejection occurred in the third.[12] For these recipients of allogeneic donor stem cells, the hypothesis that more intense immune suppressive conditioning therapies may override protection conferred by NIMA-specific tolerance is consistent with comparable survival rates of NIMA-mismatched solid organ allografts in retrospective cohorts when increased potency immune suppression with cyclosporine is used to avert rejection.[6,13] However, the lack of significant protective benefits in these isolated contexts does not negate the need for further investigating how naturally engrained NIMA-specific tolerance can be therapeutically exploited in transplantation and other clinical areas that require more stringent immunological regulation (e.g. allergy, autoimmunity, maternal-fetal tolerance).

Another more intriguing explanation for incomplete and discordant phenotypes of NIMA-specific tolerance may be related to variations in postnatal exposure to maternal cells bearing NIMA through breastfeeding, and ensuing differences in levels of maternal cell microchimerism.[14-17] Postnatal persistence of genetically foreign chimeric maternal cells in offspring was originally described in infants with severe combined immune deficiency.[18-23] In a study of 121 infants with defective T and B lymphocyte development, 40% had engrafted maternal T cells and a similar proportion developed clinically apparent GVHD caused by anti-fetal allo-immunity.[24] Similarly in cases of maternal malignancy during pregnancy, transplacental metastases of immune evasive tumor cells has been described in melanoma, lymphoblastic leukemia, and lung adenocarcinoma.[25-27] With more sensitive techniques allowing detection of potentially rarer maternal cells or their DNA (e.g., fluorescence in situ hybridization, quantitative polymerase chain reaction), microchimerism of maternal origin is increasingly recognized to occur near ubiquitously among offspring.[28-30] Maternal cell-specific DNA can be detected in up to 30% of cord blood specimens at a median concentration of 0.3% among fetal cells,[31] whereas DNA encoding maternal MHC haplotype alleles are found in 22% to 55% of healthy adult individuals after analysis of less than 2 μg of peripheral blood DNA.[28,29] Thus, vertical transfer and engraftment of maternal cells in offspring is likely an unavoidable by-product of in utero development through a porous placental interface.

The additional immune modulatory properties of breastfeeding on NIMA-specific tolerance are most definitively illustrated by improved outcomes among breast-fed individuals receiving maternal donor kidney allografts.[16] In retrospective inquires on breastfeeding, functional survival of the maternal allograft was significantly increased among breast-fed compared with non-breast fed renal transplant recipient offspring. These protective benefits of breastfeeding were specific to maternal allograft tissue bearing NIMA, since no differences in paternal donor allograft survival were identified.[16] These remarkable benefits in human transplantation strongly implicate potent antigen-specific immune modulatory properties of soluble maternal-HLA in breast milk.[32,33] In turn, direct associations between breastfeeding, increased postnatal persistence of microchimeric maternal cells and NIMA-specific tolerance shown in complementary animal studies suggest breast milk may contain a critical source of maternal cells that establish microchimerism in offspring.[14,15,17,29,33] Alternatively, another interpretation of increased maternal cell microchimerism in breast fed individuals is that NIMA-specific tolerance prevents rejection of genetically foreign cells transferred through breast milk.

Additional clues on the developmental ontogeny and adaptive immune components responsible for human NIMA-specific tolerance were unveiled in pioneering analysis of latent anti-maternal immunity in T cells of fetal and adult offspring.[34] Recognizing the need for active suppression among maturing fetal T cells during in utero development to avert potentially harmful anti-maternal immunity, Mold, McCune and colleagues showed tolerogenic fetal immune suppressive regulatory T cells develop from exposure to genetically foreign maternal alloantigens.[35] This dedicated fetal CD4+ T cell subset identified by expression of the Foxp3 transcriptional regulator or high-affinity IL-2 cytokine receptor, CD25, was shown to selectively suppress anti-maternal responses. In elegant co-culture assays between purified fetal T cells and antigen presenting cells from the biological mother or non-related adult donors, selective suppression of anti-maternal T cell proliferation by fetal CD25+ T cells was demonstrated.[35] Expansion of CD25+ or Foxp3+ regulatory T cells that suppress anti-maternal immunity also paralleled microchimeric maternal cells in fetal lymph node tissue. Thus for human infants with numerically replete T cells at the time of birth, NIMA-specific tolerance is likely essential for restraining harmful anti-maternal immunity during in utero and early postnatal

development when exposure to foreign maternal antigens is unavoidable.[34,35] With this reasoning, postnatal persistence of NIMA-specific tolerance through adulthood can be viewed as a developmental remnant of immune suppressive pathways essential for in utero fetal-maternal co-habitation. Together, this emerging body of human retrospective and experimental data sparked by Owen's initial characterization of maternal Rh ancestral phenotype highlight profound immune modulatory properties engrained through developmental NIMA exposure. However, this intriguing immunological association between NIMA-specific tolerance, maternal cell microchimerism, and expanded fetal regulatory T cells that actively suppress anti-maternal immunity also raises provocative new questions with regards to why this biological phenomenon is programmed to persist through adulthood.

Animal models of immune tolerance with early developmental antigen exposure

Nearly 10 y after Owen's seminal description of immunological tolerance between genetically discordant fraternal twin cattle, Dr. Peter Medawar's analysis of skin graft survival provided pivotally important experimental evidence supporting the existence of actively acquired tolerance to genetically foreign tissues.[36] Employing unique strains of highly inbred genetically identical mice, Medawar's classical experiments showed in utero exposure to cells from discordant mouse strains can confer tolerance to skin grafts that persists through adulthood. Tolerance to skin grafting in chickens was similarly observed after embryonic cell transfer between unique inbred strains identified by distinctive feather coloration.[36] Although the use of genetically homozygous inbred animals in these studies precludes analysis of NIMA-specific tolerance, these results nonetheless clearly established antigen-specific tolerogenic properties stemming from in utero and early developmental antigen exposure. Considering immunological tolerance to developmentally irrelevant alloantigens can be primed by in utero stimulation, physiological exposure of the fetus to semi-allogeneic maternal tissues would be expected to confer similar, if not more profound, immunological tolerance to NIMA.

There is now definitive evidence that NIMA-specific tolerance initially described in retrospective analysis of human transplantation outcomes can be faithfully reproduced and further dissected in animals. Using an elegant F1 backcross breeding strategy to generate genetically identical mice discordantly exposed to defined MHC haplotype alleles as surrogate NIMA or NIPA, Burlingham and colleagues showed remarkable protective benefits against rejection of fully allogeneic NIMA-compatible donor heart grafts.[14,37] In parallel with the aforementioned improved survival of human maternal kidney allografts in breast-fed compared with non-breast fed recipient offspring,[16] complementary cross fostering nursing studies in mice show maternal antigen exposure both in utero and through oral breast milk ingestion are simultaneously essential for improved survival of NIMA-mismatched cardiac allografts.[14]

Similarly in animal models of haematopoietic stem cell transplantation, GVHD was attenuated in irradiated recipient mice reconstituted with allogeneic NIMA-mismatched donor splenocytes.[38] Postnatal exposure to NIMA through breastfeeding also enhances protection against GVHD for immune progenitor cells transferred into NIMA-mismatched irradiated recipient mice,[15] and these beneficial impacts are directly linked with postnatal persistence of microchimeric maternal cells in offspring.[17] In turn, analysis of individual mice after solid organ or haematopoietic stem cell transplantation have identified direct associations between NIMA-tolerant phenotypes, levels of maternal cell microchimerism and expanded accumulation of CD25$^+$, Foxp3$^+$, or transforming growth factor-β producing regulatory CD4$^+$ T cells.[15,17,37,39] Thus, animal models of NIMA-specific tolerance amenable to experimental investigation have been instrumental in verifying, as well as further establishing the immunological cellular and molecular mechanisms responsible for NIMA-specific tolerance.[39]

The study of in utero transplantation of genetically foreign cells also exploits the tolerogenic properties unique to fetal development and provides important mechanistic clues on NIMA-specific tolerance.[40-42] The theoretical advantage of in utero transplantation is that therapeutic introduction of genetically foreign cells into the fetal recipient prior to maturation of adaptive immune components can induce long-term donor-specific tolerance without the need for toxic myeloablative conditioning. Animal models of in utero haematopoietic cell transplantation highlight the critical importance of a minimum threshold of antigen exposure necessary to establish and maintain

allo-specific tolerance.[40,43-48] Therefore, tolerance to NIMA that parallels persistence of maternal origin microchimeric cells also likely hinges on a minimum level of exposure to microchimeric maternal cells.[17,44] Further study is needed however, to determine how the level of maternal microchimerism may dictate alternate outcomes of autoimmunity or NIMA-specific tolerance. Additionally, given that in utero transplantation of genetically foreign cells to the fetus does not occur in isolation from the immunologically competent mother, maternal allo-sensitization can result from the introduction of discordant third-party alloantigens into the fetus.[49,50] Thus, discordance in protective benefits of NIMA-specific tolerance after transplantation may also reflect transfer of maternal adaptive immune components that have undergone sensitization to fetal-specific antigen.[6,9-13]

To more definitively identify the specificity of immune suppressive regulatory T cells that expand with developmental NIMA exposure, we developed a breeding strategy that uniquely transforms defined model antigens into surrogate NIMA.[51] Specifically, female mice heterozygous for a defined transgene that encodes cell surface expression of a recombinant protein containing ovalbumin plus the 2W1S variant of mouse I-Eα peptide were used for mating with non-transgenic males.[52,53] This approach that simultaneously transforms the MHC class II I-A^b:2W1S$_{55-68}$ peptide plus ovalbumin into surrogate NIMA in half the offspring, combined with tetramer staining and bead enrichment tools for precisely identifying rare I-A^b:2W1S-specific CD4$^+$ T cells, provided a unique opportunity to investigate the differentiation of endogenous NIMA-specific cells.[54] We found CD4$^+$ T cells with surrogate-NIMA specificity in NIMA-exposed adult mice became highly enriched (~50%) for Foxp3 expression compared with CD4$^+$ T cells of the same specificity in NIPA exposed or control mice without developmental 2W1S exposure.[51] In agreement with aforementioned human and mouse studies highlighting the importance of postnatal stimulation by genetically foreign maternal cells through breastfeeding,[15-17] expanded accumulation of NIMA-specific Foxp3$^+$ regulatory T cells declined sharply in cross fostered mice exposed to maternal tissues bearing NIMA-2W1S during in utero development or through breastfeeding in isolation. On the other hand, comparable absolute numbers of I-A^b:2W1S-specific CD4$^+$ T cells, and their similar avidity for cognate I-A^b:2W1S

peptide, between NIMA exposed and naive control mice suggest thymic deletion of NIMA responsive cells play less important roles in immunological tolerance to NIMA. (ref. 51 and unpublished data) Thus, early developmental exposure to NIMA primes in offspring an expanded pool of peripherally induced immune suppressive regulatory T cells with NIMA-specificity.

Sustained expansion of NIMA-specific regulatory T cells in offspring also paralleled postnatal retention of microchimeric maternal cells. OVA encoding DNA specific to genetically discordant maternal cells was identified in vital organs (e.g. liver, heart) of NIMA exposed mice, at levels corresponding to 1 maternal cell in 10^5 to 10^6 offspring cells in agreement with other studies using complementary tools for estimating levels of maternal microchimerism in mice and non-human primates.[17,47,48,51,55,56] One interpretation of these data is that postnatal persistence of NIMA-specific tolerance is a developmental remnant that protects genetically foreign microchimeric maternal cells from rejection in offspring. Alternatively, retained microchimeric maternal cells may have themselves adapted tolerogenic properties required for driving expanded accumulation of NIMA-specific regulatory T cells and therefore promote their own survival.

To definitively investigate the cause and effect relationship between the interrelated phenomena of expanded accumulation of NIMA-specific regulatory T cells and microchimeric maternal cells simultaneously retained in offspring, the impacts of selectively depleting microchimeric maternal cells based on co-expression of ovalbumin protein with 2W1S$_{55-58}$ peptide in NIMA exposed mice was evaluated. Remarkably, expanded accumulation of NIMA-specific regulatory T cells declined to background levels found in NIPA or naive control mice within the first 2 weeks after depleting microchimeric 2W1S-OVA$^+$ maternal cells with anti-ovalbumin antibodies.[51] These results are consistent with the hypothesis that microchimeric cells provide an essential source of cognate maternal antigen required for maintaining expanded accumulation of NIMA-specific regulatory T cells. In this regard, numerical retention of memory regulatory T cells with NIMA-specificity appear to share with effector CD4$^+$ T cells of foreign microbial specificity the necessity for frequent, if not constant, cognate antigen exposure reminders. Based on these findings, it may be worthwhile to investigate if memory regulatory T cells described in other contexts (e.g., transient

expression in the skin or after acute infection with viral pathogens).[57-60] represent *bona fide* memory like activated CD8^{+} T cells, or alternatively share a requirement for low-level exposure to cognate antigen.[61-65] In the broader scientific context, these results illustrate how dissecting the fundamental immunology responsible for NIMA-specific tolerance can continue to reveal hidden immunological secrets engrained in mammalian reproduction.

Teleological benefits and immunological consequences of NIMA-specific tolerance

Despite the primary use of animal models to verify the existence and further establish immunological mechanisms responsible for NIMA-specific tolerance, comparison of NIMA-specific tolerance across mammalian species can also provide critical insights on the evolutionary ontogeny of this highly engrained immunological phenomena. For human infants with numerically replete adaptive immune components at the time of birth, tolerance to genetically foreign maternal cells and tissues begins in utero with suppressed activation of maturing immune cells with NIMA-specificity.[34,35] However, this reasoning does not explain why tolerance imprinted by exposure to foreign antigens during early development is widely conserved across mammalian species (e.g. non-human primates, ruminants, rodents) with sharply delayed adaptive immune cell maturation relative to parturition.[34,66] For example, prolonged survival of NIMA-matched allografts and expanded NIMA-specific regulatory T cells in human adults is consistently reproduced in adult mice despite the absence of peripheral T cells at the time of birth for this species.[14,34,37] The preservation of NIMA-specific tolerance in mammalian species born without functional adaptive immune components suggests the existence of more universal biological benefits driving conserved tolerance to NIMA in placental mammals.

An important clue is postnatal persistence of NIMA-specific tolerance through adulthood that is actively maintained by maternal cells that established microchimerism in offspring.[51] In turn, given the necessity for expanded tolerance that encompasses immunologically foreign paternal-fetal antigens in successful pregnancy shared by all eutherian placental mammals,[67-69] we reasoned reinforced fetal tolerance during next-generation pregnancies may represent a more universal explanation for evolutionarily conserved NIMA-specific tolerance. This notion is supported by our recent demonstration of expanded NIMA-specific regulatory T cell accumulation in female compared with male NIMA-2W1S littermate offspring, and correspondingly enriched levels of microchimeric maternal cell DNA retained in female gender specific reproductive tissue.[51]

To further investigate this hypothesis, susceptibility to complications during allogeneic pregnancy stemming from disruptions in fetal tolerance were evaluated in genetically identical female mice developmentally exposed to discordant MHC haplotypes as surrogate NIMA. Remarkably, this analysis showed NIMA-specific tolerance confers profound resiliency against fetal wastage normally triggered by infection with the prenatal bacterial pathogen *Listeria monocytogenes* or partial transient depletion of bulk maternal regulatory T cells.[51,70-72] These protective benefits occurred in an antigen-specific fashion requiring commonality between NIMA and paternal-fetal expressed antigens since susceptibility to fetal wastage rebounded when third-party male mice bearing irrelevant MHC haplotype alleles were used for mating with NIMA-exposed female mice. Thus, expanded accumulation of regulatory T cells with fetal specificity, primed by either developmental NIMA stimulation or prior pregnancy, efficiently overrides susceptibility to invasive infection with prenatal pathogens like *Listeria monocytogenes* conferred by increased non-specific immune suppression from accumulation of bulk maternal regulatory T cells.[71,73] Given the pivotal importance of decidual infiltration by activated fetal-specific CD8^{+} T cells in the immune-pathogenesis of fetal wastage that occurs with prenatal infection,[70] dissecting the anatomical and molecular details whereby fetal-specific regulatory CD4^{+} T cells efficiently reinforce fetal tolerance are critically important areas for future investigation with direct translational implications for improving human pregnancy outcomes.

Cross-generational protection against fetal wastage in animal pregnancy models are also in agreement with the 'grandmother effect' reported by Gammill, Nelson and colleagues where reduced rates of pregnancy complications stemming from disruptions in fetal tolerance (e.g., preeclampsia, recurrent miscarriage) in women parallel increased levels of microchimeric cells retained from their mothers.[74-76] Given the

necessity for overlap between NIMA and fetal-expressed antigen during next-generation pregnancies for reinforced fetal tolerance in mice,[51] important next-steps are to investigate if similar overlap augments resiliency against complications in human pregnancy. In this regard, while developmental exposure to the single minor Rh alloantigen initially described by Owen was insufficient to prevent hemolytic disease of the newborn,[3,4] these data suggest broader non-inherited antigenic overlap between maternal grandmother and offspring that encompasses MHC haplotype alleles may more efficiently confer cross-generational reproductive benefits.

These findings also highlight striking commonality between reproductive benefits conferred by expanded regulatory T cells with NIMA-specificity retained in females from developmental exposure to genetically foreign maternal antigens, and regulatory T cells with fetal specificity retained in mothers after prior pregnancy.[73] In each case, memory regulatory T cells in reproductive age females re-accumulate with sharply accelerated tempo in response to cognate fetal antigen stimulation that protects against disruptions in fetal tolerance. The actively retained enriched pool of memory maternal regulatory T cells with specificity to pre-existing fetal antigen provides an intriguing scientific framework that explains human partner-specific protective benefits of prior pregnancy against complications in subsequent pregnancies.[77,78] Given the necessity for cognate antigen reminders in the form of microchimeric maternal cells in maintaining expanded accumulation of NIMA-specific regulatory T cells,[51] it is tantalizing to hypothesize that the protective subset of *mother's little helpers* in the form of maternal regulatory T cells with pre-existing fetal specificity are similarly maintained by fetal cells that establish persistent microchimerism in mothers after parturition.[79-83] In other words, microchimeric maternal cells that promote cross-generational reproductive fitness, and fetal cells that establish microchimerism in mothers after pregnancy can each be more accurately viewed as *mother's little genetic helpers*. Thus, establishing functional similarities and potential differences between how maternal compared with fetal microchimeric cells prime and sustain expanded memory regulatory T cells represent critically important areas for future investigation.

In the larger biological context, reproductive fitness during next generation pregnancies conferred by NIMA-specific tolerance underscores the remarkably engrained drive for genetic fitness in each female individual. In addition to transmitting half of homologous chromosomes through Mendelian inheritance, vertically transferred maternal cells that establish microchimerism in offspring selectively enforce fetal tolerance during next generation pregnancies that promotes conservation of NIMA.[51] However in nature, NIMA-specific tolerance among dominant female individuals is likely counterbalanced by pathogen-mediated selection for MHC diversity among homologous chromosomes within individuals, and between individuals across outbred populations.[30,84,85] Together, these findings highlight the need for more extended cross-generational analysis to resolve the ongoing controversy regarding how MHC haplotype similarity impacts mate selection and pregnancy outcomes.[86-88] Here, the efficiency whereby NIMA-specific tolerance retained in adult female mice protects against disruptions in fetal tolerance during next generation pregnancies strongly suggests enhanced protection against rejecting the fetal allograft in addition to other selective benefits promoting pathogen resistance drives preservation of this immunological phenomena at least in some placental mammalian species.

On the other hand, cross-generational reproductive advantages that preserve postnatal retention of microchimeric maternal cells may also perpetuate auto-inflammatory or autoimmune diseases in offspring.[89,90] These potentially harmful consequences of retained microchimeric maternal cells have been best characterized in individuals with scleroderma where increased levels of maternal microchimerism have been identified.[28,29] Enriched microchimeric maternal cells are found in the peripheral blood and pancreatic tissue of individuals with type 1 diabetes.[91] For individuals with rheumatoid arthritis, remarkable links between noninherited maternal HLA-DR alleles associated with disease susceptibility and resistance have each been described.[92-94] Similarly, maternal cell microchimerism has also been recently shown to be increased among premature offspring in animal models of inflammation-induced preterm birth.[95]

Sharply increased levels of microchimeric cells have also been described in the target tissue of infants and children with various autoimmune disorders. For example, significantly increased levels of female chimeric cells, presumably of maternal origin, were

identified in muscle biopsy specimens of children with juvenile dermatomyositis or other idiopathic inflammatory myopathies.[96,97] Maternal chimeric cells were also uniformly identified in 15 cardiac biopsy samples from infants with neonatal lupus syndrome,[98] and 2 independent case series of liver biopsy specimens from infants with biliary atresia.[94,99] Together, these clinical observations support the intriguing possibility that allo-reactivity either against or initiated by genetically foreign microchimeric cell could represent an important trigger for human autoimmune and autoinflammatory disorders.[89,100]

Interestingly however, there is also compelling data for improved survival of maternal compared with paternal hepatic allografts for infants with liver failure secondary to biliary atresia.[96,97,101] Therefore, a definitive pathological role for microchimeric maternal cells in triggering autoimmunity will require additional investigation since enriched chimeric cells in damaged or diseased tissues may also reflect their participation in tissue repair and regeneration. Furthermore, qualitative shifts in the molecular phenotype of microchimeric cells, timing and inflammatory context of developmental exposure to maternal tissues are each also likely to play profound roles in controlling whether tolerance or sensitization to NIMA develops.[39,40,44,102] Given the conserved nature of NIMA-specific tolerance and long-term retention of microchimeric maternal cells in humans and mice, further studies using representative animal models of autoimmunity that bypass limitations in human tissue availability are likely to be highly informative in dissecting the beneficial and detrimental impacts of increasingly recognized constitutive chimerism among individuals.

Concluding perspectives

Sixty years since the initial description of actively acquired tolerance to Rh antigens by Dr. Ray Owen have witnessed a dramatic explosion of new data highlighting not only the existence of NIMA-specific tolerance, but also the translational applicability of this engrained immunological phenomenon in human solid organ and stem cell transplantation. With new technology for identifying exceptionally rare microchimeric maternal cells and transgenic mouse tools for tracking NIMA-specific immune components, NIMA-specific tolerance is now recognized to occur with vertical transmission of maternal cells that establish persistent microchimerism in offspring. In turn, the recognition of individuals in outbred populations as being 'constitutively chimeric,' with non-inherited legacy of tolerogenic microchimeric cells, forces reconsideration of immunological identity beyond binary definitions of self versus non-self antigen distinction – that incorporates transmission of maternal attributes through matrilineal non-Mendelian heredity.[103] Along with improved outcomes after transplantation, further mining immunological secrets engrained within mammalian reproduction initially recognized by Owen may hold exciting new keys for more effective therapeutic strategies for preventing pregnancy complications and reversing autoimmunity.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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